TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	ATTORNEY'S DOCKET NUMBER 1581/00265
	U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/830111

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP00/05659	24 August 2000	24 August 1999

TITLE OF INVENTION

PROCESS FOR PRODUCING COENZYME Q10

APPLICANT(S) FOR DO/EO/US

MATSUDA, Hideyuki , KAWAMUKAI, Makoto , YAJIMA, Kazuyoshi , IKENAKA, Yasuhiro , HASEGAWA, Junzo , TAKAHASHI, Satomi

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. § 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a ☐ is transmitted herewith (required only if not transmitted by the International Bureau)
   - b. ☒ has been transmitted by the International Bureau.
   - c ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   - b ☐ have been transmitted by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the Annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter

16. ☒ Other items or information. Receipt in the Case of an Original Deposit, ISR, Sequence Listing (paper copy only)

U.S. APPLICATION NO (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/830111	PCT/JP00/05659	1581/00265

☒ The following fees are submitted:

	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO ..... **$860.00** International preliminary examination fee paid to USPTO (37 CFR 1.482) **$0.00**		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)). **$710.00**		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... **$1,000.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..... **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e))	**$**	

Claims	Number Filed	Number Extra	Rate	
Total Claims	20- 20 =	0	X **$18.00**	**$0**
Independent Claims	1- 3 =	0	X **$80.00**	**$0**
Multiple dependent claim(s)(if applicable)			+ **$270.00**	**$**
TOTAL OF ABOVE CALCULATIONS =				**$860**
Reduction by 1/2 for filing by small entity, if applicable.				**$**
SUBTOTAL =				**$860**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				**$**
TOTAL NATIONAL FEE =				**$860**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				**$**
TOTAL FEES ENCLOSED =				**$860**
				Amount to be: refunded **$**
				charged **$**

a. ☒ A check in the amount of $860 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. **22-0185** in the amount of $_____ to cover the above fees
A duplicate copy of this sheet is enclosed.
c. ☒ The Director is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **22-0185**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status

SEND ALL CORRESPONDENCE TO:
Connolly Bove Lodge & Hutz LLP
1990 M Street, N.W., Suite 800
Washington, DC 20036-3425


SIGNATURE

Burton A. Amernick
NAME

24,852
REGISTRATION NUMBER

#3

09/830111

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:		
Hideyuki MATSUDA et al.		
Serial No.: 09/830,111		Art Unit:
Filed:		Examiner:
For: PROCESS FOR PRODUCING COENZYME Q10		Atty Docket: 1581/00265

TRANSMITTAL OF CRF OF SEQUENCE LISTING
AND STATEMENT OF IDENTITY

Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to Form PCT/DO/EO/920 mailed in the above-captioned case on May 21, 2001, attached please find a CRF of the Sequence Listing. The contents of the paper copy of the Sequence Listing filed on April 24, 2001 and this CRF of the Sequence Listing are identical and includes no new matter.

Respectfully submitted,

July 23, 2001

FOR Burton A. Amernick (24,852)
Connolly Bove Lodge & Hutz LLP
1990 M Street, N.W.
Washington, D.C. 20036-3425
Telephone: 202-331-7111

Date:

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	:	
Hideyuki MATSUDA et al.	:	
Serial No.: To be assigned	:	Art Unit: To be assigned
Filed: Herewith	:	Examiner: To be assigned
For: PROCESS FOR PRODUCING COENZYME Q10	:	Atty Docket: 1581/00265

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to initial examination, please amend the above-captioned case as follows.

IN THE CLAIMS

Please amend the claims as follows.

4. (Amended) An expression vector constructed by cloning the DNA according to Claim 1 in an expression vector.

7. (Amended) A transformant as obtainable by transforming a host microorganism with the DNA according to Claim 1.

8. (Amended) A transformant as obtainable by transforming a host microorganism using the expression vector according to Claim 4.

9. (Amended) The transformant according to Claim 7 wherein the host microorganism is *Escherichia coli*.

12. (Amended) A process for producing a coenzyme Q_{10} which comprises culturing the transformant according to Claim 7 in a culture broth and harvesting the coenzyme Q_{10} produced and accumulated in the resulting culture.

Please add the following new claims.

13. (New) An expression vector constructed by cloning the DNA according to Claim 3 in an expression vector.

14. (New) A transformant as obtainable by transforming a host microorganism with the DNA according to Claim 3.

15. (New) A transformant as obtainable by transforming a host microorganism using the expression vector according to Claim 5.

16. (New) A transformant as obtainable by transforming a host microorganism using the expression vector according to Claim 6.

17. (New) The transformant according to Claim 8 wherein the host microorganism is *Escherichia coli*.

18. (New) A process for producing a coenzyme Q_{10} which comprises culturing the transformant according to Claim 8 in a culture broth and harvesting the coenzyme Q_{10} produced and accumulated in the resulting culture.

19. (New) A process for producing a coenzyme Q_{10}

which comprises culturing the transformant according to Claim 9 in a culture broth

and harvesting the coenzyme Q_{10} produced and accumulated in the resulting culture.

20. (New) A process for producing a coenzyme Q_{10}

which comprises culturing the transformant according to Claim 10 in a culture broth

and harvesting the coenzyme Q_{10} produced and accumulated in the resulting culture.

REMARKS

The claims have been amended to eliminate multiple dependency and to improve their format. None of these amendments is believed to involve any new matter. Accordingly, it is respectfully requested that the foregoing amendments be entered, that the application as so amended receive an examination on the merits, and that the claims as now presented receive an early allowance.

Respectfully submitted,

Burton A. Amernick (24,852)
Connolly Bove Lodge & Hutz LLP
1990 M Street, N.W., Suite 800
Washington, D.C. 20036-3425
Telephone: 202-331-7111

Date: 4-24-01

APPENDIX – MARKED UP VERSION

4. (Amended) An expression vector constructed by cloning the DNA according to Claim 1 [or 3] in an expression vector.

7. (Amended) A transformant as obtainable by transforming a host microorganism with the DNA according to Claim 1 [or 3].

8. (Amended) A transformant as obtainable by transforming a host microorganism using the expression vector according to Claim 4[, 5 or 6].

9. (Amended) The transformant according to Claim 7 [or 8] wherein the host microorganism is *Escherichia coli*.

12. (Amended) A process for producing a coenzyme Q_{10}

which comprises culturing the transformant according to Claim 7[, 8, 9, 10 or 11] in a culture broth

and harvesting the coenzyme Q_{10} produced and accumulated in the resulting culture.

#7

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Conf. No.: 3002
Hideyuki MATSUDA et al.	
Serial No.: 09/830,111	
Int'l FD: August 24, 2000	
For: PROCESS FOR PRODUCING COENZYME Q10	Atty Docket: 21581/0265

RESPONSE AND AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Notification of Missing Requirements dated February 19, 2002 (copy attached), attached are substitute paper and CRF copies of the sequence listing. These do not introduce any new matter. The contents of these paper and CRF copies of the sequence listing are identical.

Please enter the paper copy of the sequence listing into the specification.

In view of the above, consideration and allowance are, therefore, respectfully solicited.

In the event the Examiner believes an interview might serve to advance the prosecution of this application in any way, the undersigned attorney is available at the telephone number noted below.

The Director is hereby authorized to charge any fees, or credit any overpayment, associated with this communication, including any extension fees, to CBLH Deposit Account No. 22-0185.

Respectfully submitted,

Burton A. Amernick (24,852)
Customer Number 30678
Connolly Bove Lodge & Hutz LLP
1990 M Street, N.W., Suite 800
Washington, D.C. 20036-3425
Telephone: 202-331-7111

Date: 4-19-02

Sequence listing

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<151> 1999-08-24

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aaatccatat acc atg gcc tca cca gca ctg cgg ata cga agc atc agc 169
Met Ala Ser Pro Ala Leu Arg Ile Arg Ser Ile Ser
1 5 10

tct cga tca atc gcc tct ctg cga tcg gtt acc cta aga aca gcc tcg 217
Ser Arg Ser Ile Ala Ser Leu Arg Ser Val Thr Leu Arg Thr Ala Ser
15 20 25

gca cct tca tta cga cta aga tgt acc ccg acg agc cgg cca tcg agt 265
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tca tgg gct gct gct gtg tct tcg gcg tcg aga ctg gtt gag cct gat 313
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ccg aat caa cct ctc atc aat ccg ctc aac ttg gtc ggt ccc gag atg 361
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65 70 75

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        335                 340                 345

ggt gca gac ctc cag ctc ggt ctc gcc acc gcg ccg gcc ctc ttc gca   1225
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                385                 390                 395

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PCT09

RAW SEQUENCE LISTING DATE: 06/04/2002
PATENT APPLICATION: US/09/830,111A TIME: 16:01:21

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Output Set: N:\CRF3\06042002\I830111A.raw

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 42                     65                  70                  75
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 57 Pro Lys Val Gln Gly Trp Glu Lys Val Val Glu Val Pro Val Asn Glu
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RAW SEQUENCE LISTING DATE: 06/04/2002
PATENT APPLICATION: US/09/830,111A TIME: 16:01:21

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Output Set: N:\CRF3\06042002\I830111A.raw

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65 Asn Met Arg Ser Gly Pro Leu Thr Lys Asp Gly Glu Ile Glu Gly Gln
66              160                 165                 170
68 acg tcg aat atc ctc gcc tcg caa cgg cgg ttg gct gag atc acg gag    697
69 Thr Ser Asn Ile Leu Ala Ser Gln Arg Arg Leu Ala Glu Ile Thr Glu
70          175                 180                 185
72 atg atc cat gca gca tca ctc ctc cac gac gac gtt atc gac gct tcc    745
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74      190                 195                 200
76 gag acc aga cga aac gca cca tcc gga aac cag gca ttc gga aac aag    793
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78 205                 210                 215                 220
80 atg gcg att ttg gct ggt gat ttc ttg ttg gga cgg gcg tct gtt gca    841
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85 Leu Ala Arg Leu Arg Asn Pro Glu Val Ile Glu Leu Leu Ala Thr Val
86              240                 245                 250
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89 Ile Ala Asn Leu Val Glu Gly Glu Phe Met Gln Leu Lys Asn Thr Val
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93 Asp Asp Ala Ile Glu Ala Thr Ala Thr Gln Glu Thr Phe Asp Tyr Tyr
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96 ttg cag aag act tac ttg aag act gcg tcc ttg att gcc aag tcg tgc   1033
97 Leu Gln Lys Thr Tyr Leu Lys Thr Ala Ser Leu Ile Ala Lys Ser Cys
98 285                 290                 295                 300
100 aga gca agt gcg ctt ctg ggt ggt gct acg cct gag gtt gct gat gct  1081
101 Arg Ala Ser Ala Leu Leu Gly Gly Ala Thr Pro Glu Val Ala Asp Ala
102                 305                 310                 315
104 gct tat gct tac gga agg aac ctt ggt ttg gca ttc cag atc gtc gac  1129
105 Ala Tyr Ala Tyr Gly Arg Asn Leu Gly Leu Ala Phe Gln Ile Val Asp
106             320                 325                 330
108 gac atg ctc gac tac acc gtc tcc gct acc gac ctc ggt aag ccc gcc  1177
109 Asp Met Leu Asp Tyr Thr Val Ser Ala Thr Asp Leu Gly Lys Pro Ala
110         335                 340                 345
112 ggt gca gac ctc cag ctc ggt ctc gcc acc gcg ccg gcc ctc ttc gca  1225
113 Gly Ala Asp Leu Gln Leu Gly Leu Ala Thr Ala Pro Ala Leu Phe Ala
114     350                 355                 360
116 tgg aag cac cac gcc gag ctc ggt ccc atg atc aag cgc aag ttc tct  1273
117 Trp Lys His His Ala Glu Leu Gly Pro Met Ile Lys Arg Lys Phe Ser
118 365                 370                 375                 380
120 gac cca gga gac gtc gag cgt gca cgc gag ttg gtc gag aaa agt gat  1321
121 Asp Pro Gly Asp Val Glu Arg Ala Arg Glu Leu Val Glu Lys Ser Asp
122                 385                 390                 395
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RAW SEQUENCE LISTING DATE: 06/04/2002
PATENT APPLICATION: US/09/830,111A TIME: 16:01:22

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134      430                 435                 440
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138 atgagagaag attttcagcc tgatacagat taaatcagaa cgcagaagcg gtctgataaa 1587
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142 agtgaa                                                          1653
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146 <211> LENGTH: 440
147 <212> TYPE: PRT
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150 <400> SEQUENCE: 2
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153 Ile Ala Ser Leu Arg Ser Val Thr Leu Arg Thr Ala Ser Ala Pro
154               20                  25                  30
155 Ser Leu Arg Leu Arg Cys Thr Pro Thr Ser Arg Pro Ser Ser Ser
156           35                  40                  45
157 Trp Ala Ala Ala Val Ser Ser Ala Ser Arg Leu Val Glu Pro Asp
158           50                  55                  60
159 Pro Asn Gln Pro Leu Ile Asn Pro Leu Asn Leu Val Gly Pro Glu
160           65                  70                  75
161 Met Ser Asn Leu Thr Ser Asn Ile Arg Ser Leu Leu Gly Ser Gly
162           80                  85                  90
163 His Pro Ser Leu Asp Thr Val Ala Lys Tyr Tyr Val Gln Ser Glu
164           95                 100                 105
165 Gly Lys His Ile Arg Pro Leu Met Val Leu Leu Met Ala Gln Ala
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167 Thr Glu Val Ala Pro Lys Val Gln Gly Trp Glu Lys Val Val Glu
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176          185                 190                 195
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178          200                 205                 210
179 Pro Ser Gly Asn Gln Ala Phe Gly Asn Lys Met Ala Ile Leu Ala
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RAW SEQUENCE LISTING DATE: 06/04/2002
PATENT APPLICATION: US/09/830,111A TIME: 16:01:22

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189 Gln Lys Thr Tyr Leu Lys Thr Ala Ser Leu Ile Ala Lys Ser Cys
190                 290                 295                 300
191 Arg Ala Ser Ala Leu Leu Gly Gly Ala Thr Pro Glu Val Ala Asp
192                 305                 310                 315
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196                 335                 340                 345
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200                 365                 370                 375
201 Lys Arg Lys Phe Ser Asp Pro Gly Asp Val Glu Arg Ala Arg Glu
202                 380                 385                 390
203 Leu Val Glu Lys Ser Asp Gly Leu Glu Lys Thr Arg Ala Leu Ala
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205 Glu Glu Tyr Ala Gln Lys Ala Leu Asp Ala Ile Arg Thr Phe Pro
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VERIFICATION SUMMARY DATE: 06/04/2002
PATENT APPLICATION: US/09/830,111A TIME: 16:01:23

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L:9 M:270 C: Current Application Number differs, Replaced Current Application No
L:9 M:271 C: Current Filing Date differs, Replaced Current Filing Date

SPECIFICATION

PROCESS FOR PRODUCING COENZYME Q_{10}

TECHNICAL FIELD

The present invention relates to a process for producing a coenzyme Q_{10} for pharmaceutical and other uses. More particularly, the invention relates to a process for producing coenzyme Q_{10} which comprises isolating a gene coding for the coenzyme Q_{10} side-chain synthase, which is a key enzyme involved in the biosynthesis of coenzyme Q_{10}, i.e. decaprenyl diphosphate synthase, from a fungal strain of the genus Saitoella and introducing it into a host microorganism to let it elaborate coenzyme Q_{10}.

BACKGROUND ART

The conventional technology for commercial production of coenzyme Q_{10} comprises isolating the coenzyme from a tobacco or other plant and modifying the length of its side chain by a synthetic technique.

While it is known that coenzyme Q_{10} is produced by a broad spectrum of organisms ranging from microorganisms, such as bacteria and yeasts, to higher animals and plants, the method comprising culturing a microorganism and extracting coenzyme Q_{10} from the microorganism is regarded as one of the most effective production methods and has actually been exploited commercially. However, the prior art methods are invariably poor in productivity, providing for only low outputs and/or involving time-consuming procedures.

The pathways for biosynthesis of coenzyme Q_{10} in organisms are partly different between the prokaryote and the eukaryote but invariably comprise a complicated cascade of reactions involving many kinds of enzymes. However, these pathways are basically comprised of three fundamental steps, namely the step of synthesizing decaprenyl diphosphate as the precursor of the

prenyl side-chain of coenzyme Q_{10}, the step of synthesizing p-hydroxybenzoic acid as the basis of the quinone ring of coenzyme Q_{10}, and the step of coupling these two compounds together and effecting a serial substituent transformation to complete coenzyme Q_{10}. Of these reactions, the reaction determinant of the length of the side-chain of coenzyme Q_{10} and acknowledged to be the rate-determining step of its biosynthesis, i.e. the reaction catalyzed by decaprenyl diphosphate synthase, is considered to be the most important reaction. Therefore, in order that coenzyme Q_{10} may be produced with good efficiency, it seems worthwhile to isolate the key gene involved in said biosynthesis, namely the gene coding for decaprenyl diphosphate synthase, and utilize it for enhanced production of the enzyme. As sources of the gene, fungi capable of producing coenzyme Q_{10} in comparatively large amounts can be regarded as useful candidates.

Heretofore, genes coding for decaprenyl diphosphate synthase have been isolated from several kinds of microorganisms, such as Schizosaccharomyces pombe (JP09-173076A) and Gluconobacter suboxydans (JP10-57072A), etc., but the inherent coenzyme Q_{10} productivity of these microorganisms cannot be considered high enough and neither an efficient cultural protocol for these microorganisms nor an efficient isolation and purification procedure has been established as yet. Therefore, there has been a standing demand for isolation of a coenzyme Q_{10}-encoding gene from a microorganism capable of highly producing a coenzyme Q_{10}.

Devoted to providing a solution to the above-mentioned production problems, the present invention has for its object to isolate a gene coding for the enzyme synthesizing the coenzyme Q_{10} side chain from a fungal strain of the genus Saitoella and exploit it to advantage for the efficient microbial production of coenzyme Q_{10}.

DISCLOSURE OF THE INVENTION

To accomplish the above object, in the present invention, the key gene involved in the biosynthesis of coenzyme Q_{10}, namely the gene coding for decaprenyl diphosphate synthase, was isolated from a fungal strain of the genus Saitoella in the first place. Then, this gene was introduced and allowed to be expressed in a host microorganism, such as Escherichia coli, to thereby enable the host to produce coenzyme Q_{10} with efficiency.

The inventors of the present invention made intensive investigations for isolating such genes coding for decaprenyl diphosphate synthase from fungal strains of the genus Saitoella capable of producing comparatively large amounts of coenzyme Q_{10} and have succeeded in isolating said genes.

The present invention, therefore, is concerned with a DNA of the following (a), (b) or (c).

(a) a DNA having the nucleotide sequence shown under SEQ ID NO:1

(b) a DNA having a nucleotide sequence derived from the nucleotide sequence of SEQ ID NO:1 by the deletion, addition, insertion and/or substitution of one or a plurality of nucleotides

and coding for a protein having decaprenyl diphosphate synthase activity

(c) a DNA which hybridizes with the DNA having the nucleotide sequence of SEQ ID NO:1 under a stringent condition

and codes for a protein having decaprenyl diphosphate synthase activity.

The present invention is further concerned with a protein of the following (d) or (e).

(d) a protein having the amino acid sequence shown under SEQ ID NO:2

(e) a protein having an amino acid sequence derived from the amino acid sequence of SEQ ID NO:2 by the deletion, addition, insertion and/or substitution of one or a plurality of amino acids

and having decaprenyl diphosphate synthase activity.

The invention is further concerned with a DNA coding for this protein.

The present invention is further concerned with an expression vector containing said DNA. For the expression vector of the invention, various vector systems heretofore known can be utilized and, therefore, may for example be pNTSa1 as constructed by cloning the DNA having the sequence of SEQ ID NO:1 into the vector pUCNT for expression.

The present invention is further concerned with a transformant as constructed by transforming a host microorganism with said DNA. As the host microorganism for the invention, Escherichia coli can be used with advantage.

The invention is further concerned with a process for producing coenzyme Q_{10}

which comprises culturing said transformant in a culture broth and harvesting the coenzyme Q_{10} produced and accumulated in the resulting culture. The host microorganism for this process is not particularly restricted but may be Escherichia coli to mention a preferred example. The coenzyme Q produced by Escherichia coli is coenzyme Q_8 but the invention enables this microorganism to produce coenzyme Q_{10}.

The inventors made intensive investigations on the isolation of the enzyme gene from a fungal strain which belongs to the genus Saitoella and is capable of producing comparatively large amounts of coenzyme Q_{10} and succeeded in acquiring a fragment of the particular gene by a PCR technique.

The inventors compared the sequence of the known gene coding for decaprenyl diphosphate synthase with the genes cording for polyprenyl diphosphate synthases, namely long-chain prenyl synthases which are analogous to said known enzyme gene but differ from the same in chain length and, for the region of high homology, synthesized various PCR primers. Using these primers in various combinations, they studied PCR conditions. As a result, they found by analysis of the gene sequence that

when a PCR using DPS-1 (5'-AAGGATCCTNYTNCAYGAYGAYGT-3') and DPS-1 1AS (5'-ARYTGNADRAAYTCNCC-3') [in the above sequences, R means A or G; Y means C or T, and N means G, A, T or C] as primers is carried out according to the protocol of heat-treatment at 94 ℃ × 3 minutes, followed by 40 cycles of 94 ℃, 1 minute→ 43 ℃, 2 minutes→ 72 ℃, 2 minutes, a ca 220 bp fragment of the enzyme gene can be amplified from the chromosome gene of Saitoella complicata IFO 10748, a fungus belonging to the genus Saitoella.

Then, to acquire the full length of this enzyme gene, the chromosome gene of Saitoella complicata IFO 10748 is digested with the restriction enzyme EcoRI and inserted into a λ phage vector to construct a recombinant phage library. After the plaque is transferred to a nylon membrane, the plaque hybridization is carried out using the labeled PCR fragment, whereby a clone having the full-length decaprenyl diphosphate synthase gene can be obtained.

Sequencing of the decaprenyl diphosphate synthase gene occurring in the above clone reveals that the gene has the nucleotide sequence shown under SEQ ID NO:1 of SEQUENCE LISTING. The amino acid sequence deduced from the above nucleotide sequence is shown under SEQ ID NO:2. Here, a sequence characteristic of a gene coding for decaprenyl diphosphate synthase is observed.

The DNA of the invention may be any of the DNA having the nucleotide sequence shown under SEQ ID NO:1, the DNA having a nucleotide sequence derived from the sequence of SEQ ID NO:1 by the deletion, addition, insertion and/or substitution of one or a plurality of nucleotides and coding for a protein having decaprenyl diphosphate synthase activity, and the DNA which hybridizes with the DNA having the nucleotide sequence of SEQ ID NO:1 under a stringent condition and codes for a protein having decaprenyl diphosphate synthase activity.

The "nucleotide sequence derived by the deletion, addition, insertion and/or substitution of one or a plurality

of nucleotides" means any nucleotide sequence derived by the deletion, addition, insertion and/or substitution of a number of nucleotides of the order which can be deleted, added, inserted and/or substituted by the methods well known in the art, for example as described in, inter alia, Protein, Nucleic Acid, Enzyme, Supplemental Issue: Gene Amplification PCR Technology TAKKAJ 35 (17), 2951-3178 (1990) and Henry A. Erlich (ed.), PCR Technology (the translation edited by Ikunoshin Kato) (1990).

As used in this specification, the term "protein having decaprenyl diphosphate synthase activity" means a protein capable of synthesizing decaprenyl diphosphate in a yield of not less than 10%, preferably not less than 40%, more preferably not less than 60%, still more preferably not less than 80%, relative to the protein having the amino acid sequence shown under SEQ ID NO:2. Such yield measurements can be made by the technique which comprises reacting FDP (farnesyl diphosphate) and ^{14}C-IPP (radiolabeled isopentenyl diphosphate) with the enzyme of interest, hydrolyzing the resulting ^{14}C-DPP (decaprenyl diphosphate) with phosphatase, fractionating the hydrolysate by TLC, and assaying the amounts taken up in spots corresponding to the respective chain lengths (Okada et al., Eur. J. Biochem., 255, 55 to 59).

The "DNA which hybridizes with the DNA having the nucleotide sequence of SEQ ID NO:1 under a stringent condition" means a DNA obtained by colony hybridization, plaque hybridization, Southern hybridization or the like hybridization technique using the DNA having the nucleotide sequence of SEQ ID NO:1 as the probe. Anyone skilled in the art may easily acquire the objective DNA by carrying out said hybridization according to the methods described in Molecular Cloning, 2nd Edition (Cold Spring Harbor Laboratory Press, 1989).

The protein of the present invention may have the amino acid sequence shown under SEQ ID NO:2 or an amino acid sequence

derived from the amino acid sequence shown under SEQ ID NO:2 by the deletion, addition, insertion and/or substitution of one or a plurality of amino acids and having decaprenyl diphosphate synthase activity.

"The amino acid sequence derived by the deletion, addition, insertion and/or substitution of one or a plurality of amino acids" can be obtained by effecting such deletion, addition, insertion and/or substitution by the technology well known in the art, such as a region-specific mutagenesis technique. Specific procedures are described in Nucleic Acid Res. 10, 6487 (1982), Methods in Enzymology, 100, 448 (1983) and other literature.

The protein of the present invention preferably has an amino acid sequence showing a homology of not less than 60%, preferably not less than 70%, more preferably not less than 80%, still more preferably not less than 90%, further still more preferably not less than 95%, to the amino acid sequence shown under SEQ ID NO:2.

The "homology" is calculated by aligning two nucleotide sequences to be compared in the optimum format, counting the matched base positions (A, T, C, G, U or I) between the two sequences, dividing the count by the total number of bases compared, and multiplying the product by 100. Specifically, this calculation can be made using an analytical software such as Hitachi Soft Engineering's DNASIS, Software Development's GENETYX, or Finland CSC's Clustal X, for instance.

While the gene coding for decaprenyl diphosphate synthase must be ligated downstream of a suitable promoter for expression, an expression vector can be constructed, for example by excising a DNA fragment containing the gene with a restriction enzyme or amplifying the enzyme-encoding gene selectively by PCR, followed by cloning it into a vector having a promoter. In the present invention, the expression vector into which the DNA coding for the protein having decaprenyl diphosphate synthase activity may be inserted is not particularly restricted but may

for example be one constructed by ligating a suitable promoter to a plasmid derived from E. coli. The plasmid of E. coli origin includes pBR322, pBR325, pUC19 and pUC119, while the promoter includes T7 promoter, trp promoter, tac promoter, lac promoter and λPL promoter. Further, as the expression vector of this invention, pGEX-2T, pGEX-3T, pGEX-3X (all from Pharmacia), pBluescript, pUC19 (from Toyobo), pMALC2, pET-3T and pUCNT (described in WO 94/03613), etc. can also be mentioned. Among these, pUCNT can be used with advantage. To mention a specific example, the vector pNTSa1 for the expression of a decaprenyl diphosphate synthase gene can be constructed by inserting the gene having the DNA sequence shown under SEQ ID NO:1 into the expression vector pUCNT.

Then, this enzyme gene expression vector is introduced into a suitable microorganism, whereby the microorganism is rendered capable of producing coenzyme Q_{10}. The host microorganism is not particularly restricted but Escherichia coli can be used with advantage. The Escherichia coli is not particularly restricted but includes such strains as XL1-Blue, BL-21, JM109, NM522, DH5α, HB101 and DH5, among others. Among these, E. coli DH5α can be used with particular advantage. For example, when the expression vector pNTSa1 containing the decaprenyl diphosphate synthase gene is introduced into this E. coli strain, the coenzyme Q_{10}, which the intact E. coli inherently does not produce, can be produced in a large amount. This E. coli DH5α (pNTSa1) has been deposited with National Institute of Bioscience and Human-Technology (Higashi 1-1-3, Tsukuba-shi, Ibaraki, Japan) under the accession number of FERM BP-6844.

Furthermore, Escherichia coli KO229 (Journal of Bacteriology, 179, 3058-3060 (1997), the octaprenyl diphosphate synthase gene-knockout E. coli strain constructed by Kawamukai et al. as the host microorganism, is incapable of producing coenzyme Q_8 and can be utilized as the host for higher production of coenzyme Q_{10}.

The gene can be used not only singly but may be introduced together with another biosynthesis-related gene into a microorganism to thereby obtain still more satisfactory results.

Coenzyme Q_{10} can be produced by culturing the transformant obtained according to the invention and harvesting the product coenzyme Q_{10} in a per se known manner. When the host microorganism is a strain of Escherichia coli, either LB broth or M9 broth containing glucose and casamino acids can be used as the culture broth. In order that the promoter may be allowed to function with efficiency, the broth may be supplemented with a certain chemical such as isopropyl-thiogalactoside or indolyl-3-acrylic acid. Culture can be carried out at 37℃ for 17 to 24 hours, for instance, optionally under aeration or agitation. In the practice of the invention, the product coenzyme Q_{10} may be used after purification or as it is in the crude form, depending on the intended use. Isolation of coenzyme Q_{10} from the culture can be made by using known separation and purification procedures in a suitable combination. As such known separation and purification procedures, there can be mentioned techniques utilizing solubilities, such as salting-out and solvent precipitation; techniques chiefly utilizing differences in molecular weight, such as dialysis, ultrafiltration, gel filtration and (SDS-)polyacrylamide gel electrophoresis; techniques utilizing differences in charge, such as ion exchange chromatography; techniques utilizing specific affinity, such as affinity chromatography; techniques utilizing differences in hydrophobicity, such as reversed-phase high performance liquid chromatography; and techniques utilizing differences in isoelectric point, such as isoelectric focusing, among others.

The use for the coenzyme Q_{10} obtained according to the invention is not particularly restricted but the enzyme can be applied to pharmaceuticals with advantage.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a restriction map of the pNTSa1 vector containing the decaprenyl diphosphate synthase gene.

Fig. 2 is an HPLC chart of the coenzyme Q_{10} produced by the recombinant Escherichia coli DH5α as transformed with the decaprenyl diphosphate synthase gene.

Fig. 3 is an HPLC chart of the coenzyme Q_{10} produced by the recombinant Escherichia coli KO229 as transformed with the decaprenyl diphosphate synthase gene.

BEST MODE FOR CARRYING OUT THE INVENTION

(Example 1)

The chromosome DNA of Saitoella complicata IFO 10748 was prepared by the method of C. S. Hoffman et al. (Gene, 57 (1987), 267-292). Based on the homology to the known long-chain prenyl diphosphate synthase genes, PCR primers, i.e. DPS-1 (5'-AAGGATCCTNYTNCAYGAYGAYGT-3') and DPS-1 1AS (5'-ARYTGNADRAAYTCNCC-3'), were designed. In the above sequences, R stands for A or G; Y for C or T; and N for G, A, T or C. Using these primers, PCR was carried out under the conditions of heat treatment at 94 ℃, 3 min. followed by 40 cycles of 94 ℃, 1 min. → 43 ℃, 2 min. → 72 ℃, 2 min., and the PCR product was analyzed by 1.2% agarose gel electrophoresis.

The ca 220 bp fragment thus obtained was excised from the gel and purified using a DNA extraction kit (Sephaglas™ BrandPrep Kit, Amersham Pharmacia Biotech). Then, using a PCR product direct cloning kit (pT7BlueT-Vector Kit, NOVAGEN), the DNA was cloned into the E. coli expression vector to give pT7-SaDPS. Then, using a DNA sequencer (Model 377, Perkin-Elmer) and a DNA sequencing kit (Perkin-Elmer; ABI PRISM™ BigDye™ Terminator Cycle Sequence Ready Reaction Kit with AmptiTaq™ DNA Polymerase, FS), DNA sequencing was carried out according to the kit manufacturer's protocol. As a result, there was obtained a sequence corresponding to the nucleotides 717 through 924 of SEQ ID NO:1 under SEQUENCE LISTING. The

translation sequence thus obtained contained "GDFLLGRA" which is a characteristic region of polyprenyl diphosphate synthases and, therefore, was considered to be part of the decaprenyl diphosphate synthase gene.

(Example 2)

Using 0.03 μg of a pT7-SaDPS vector containing a 220 bp DNA fragment which was considered to be the decaprenyl diphosphate synthase gene of Saitoella complicata IFO 10748, PCR using primers Sa-1S (which has the sequence of 5'-GAGACCAGACGAAACGCACCA-3') and Sa-2AS (which has the sequence of 5'-TGGTGCGTTTCGTCTGGTCTC-3') was carried out [94 ℃, 3 min. → (94 ℃, 30 sec. → 55 ℃, 30 sec. → 72 ℃, 1 min.) × 25 cycles → 72 ℃, 5 min. → 4 ℃]. The PCR product was subjected to gel electrophoresis using 1.2% agarose (Takara) and a ca 145 bp fragment was excised from the gel and purified using a DNA extraction kit (Sephaglas™ BrandPrep Kit; Amersham Pharmacia Biotech). Using about 100 ng of this DNA fragment, chemiluminescence labeling was performed using ECL Direct Nucleic Acid Labeling System (Amersham Pharmacia Biotech).

(Example 3)

The chromosome DNA of Saitoella complicata IFO 10748 was digested with the restriction enzyme EcoRI and electrophoresed through 0.8% agarose gel. This gel was denatured with alkali (0.5 M NaOH, 1.5 M NaCl) and neutralized (0.5 M Tris-HCl (pH 7.5), 1.5 M NaCl), after which HYBOND N + filter (Amersham) was placed on the gel and the Southern transfer was carried out using 20 × SSC overnight. This filter was dried and heated at 80 ℃ for 2 hours and using ECL Direct Nucleic Acid Labeling/Detection System (Amersham Pharmacia Biotech), Southern hybridization and detection were carried out. Thus, using Gold Hybridizatioon Solution (Amercham Pharmacia Biotech), prehybridization was performed at 42 ℃ for 1 hour.

The chemiluminescence-labeled probe was heated at 95 ℃

for 5 minutes, quenched on ice, and added to the prehybridization solution used for filter prehybridized and the hybridization was carried out at 42 ℃ for 22 hours. This filter was washed with 0.5 × SSC solution containing 6 M urea and 0.4% SDS at 42 ℃ twice for 20 minutes each and, then, washed with 2 × SSC solution at room temperature twice for 5 minutes each. This filter was immersed in Enhanced Chemiluminescence Reagent (product of Amersham Pharmacia Biotech) and, then, exposed in intimate contact with X-ray film to detect a black exposure band.

As a result, the probe was found to have firmly hybridized with a ca. 10 kbp EcoRI restriction fragment.

(Example 4)

The chromosome DNA of Saitoella complicata IFO 10748 was digested with the restriction enzyme EcoRI and electrophoresed through 0.8% agarose and a ca. 10 kbp fragment of the DNA was excised from the gel and purified to prepare a DNA fragment for cloning. Using λ-DASHII Phage Kit (product of Stratagene), the above DNA fragment was inserted into the EcoRI site of its phage and the packaging was made using In Vitro Packaging Kit (Amersham). Escherichia coli XL1-Blue MRA (P2) was infected and layered on NZY plate medium (5 g/L NaCl, 2 g/L $MgSO_4 \cdot 7H_2O$, 5 g/L yeast extract, 10 g/L NZ amine, 18 g/L agar (pH 7.5)) together with NZY soft agar (the agar only of NZY plate medium, 8 g/L) for use as a plaque. This was transferred to HYBOND N + filter (product of Amersham), denatured with alkali (0.5 M NaOH, 1.5 M NaCl), neutralized (0.5 M Tris-HCl (pH 7.5), 1.5 M NaCl), dried, and heated at 80 ℃ for 2 hours.

Using 9 filters heated as above, the prehybridization and the hybridization using the chemiluminescence-labeled probe were carried out as in Example 3 and the filters were rinsed. Each filter was then dried and exposed in intimate contact with X-ray film and the phage plaque corresponding to the black exposure spot was separated. The phage of the separated plaque

was used to infect E. coli in the same manner as above and transferred to the filter and the hybridization was carried out again for confirmation. As a result, 6 phage clones could be selected.

Using a suspension of the phage, PCR was carried out using said Sa-1S and Sa-2AS primers, and as a result, a 145-bp DNA fragment could be detected in 6 clones. Therefore, the phage DNA was prepared from the recombinant λ-DASHII phage particles according to Laboratory Manual for Genetic Engineering (Masami Muramatsu, Maruzen, 1990). For subcloning, the phage DNA thus prepared was digested with the restriction enzymes SalI and SacI and electrophoresed through 0.8% agarose gel. This gel was denatured with alkali (0.5 M NaOH, 1.5 M NaCl) and neutralized (0.5 M Tris-HCl (pH 7.5), 1.5 M NaCl). Then, a HYBOND N + filter (Amersham) was placed on the gel and subjected to Southern transfer using 20 × SSC overnight. This filter was dried and heated at 80 ℃ for 2 hours, after which the Southern hybridization and detection were carried out using ECL Direct Nucleic Acid Labeling/Detection System (Amersham Pharmacia Biotech). Thus, using Gold Hybridizatioon Solution (Amersham Pharmacia Biotech), the prehybridization was carried out at 42 ℃ for 1 hour.

The chemiluminescence-labeled probe was heated at 95 ℃ for 5 minutes, quenched on ice, and added to the prehybridization solution used for filter prehybridized and the hybridization was carried out at 42 ℃ for 22 hours. This filter was washed with 0.5 × SSC containing 6 M urea and 0.4% SDS at 42 ℃ twice for 20 minutes each and, then, with 2 × SSC at room temperature twice for 5 minutes each. The filter was immersed in Enhanced Chemiluminescence Reagent (Amersham Pharmacia Biotech) and exposed in intimate contact with X-ray film to detect a black exposure band.

As a result, the probe was found to have intimately hybridized with a ca. 4.5 kb fragment as obtained by digestion with the restriction enzyme SalI and a ca. 3.5 kb fragment as

obtained by digestion with SacI.

The phage DNA was digested with the restriction enzymes SalI and SacI and electrophoresed through 0.8% agarose gel. The restriction fragment corresponding to the position and size of the black exposure band was excised from the gel and purified using a DNA extraction kit (Sephaglas™ Brand Prep Kit; Amersham Pharmacia Biotech). Then, using a DNA sequencer (Model 377, Perkin-Elmer Corp.) and a DNA sequence kit (Perkin-Elmer Corp., ABI PRISM™ BigDye™ Terminator Cycle Sequence Ready Reaction Kit with AmptiTaq™ DNA polymerase, FS), the sequencing was carried out in accordance with the manufacturer's protocol.

As a result, it was found that the SalI site and SacI site are located at positions 1124 and 1241, respectively, of SEQ ID NO:1 under SEQUENCE LISTING and that neither fragment contained the C-terminal. So, for SalI, which is the upstream one of the two restriction enzymes, in the decaprenyl diphosphate synthase gene, the remaining fragments were examined. As a result, a 3 kbp fragment was found to contain a sequence including a terminal region of the SacI fragment up to the termination codon. By analyzing these 3 restriction fragments, the full-length sequence of the decaprenyl diphosphate synthase gene could be elucidated. Of the three DNA fragments, the ca 1.6 kbp fragment was sequenced. The result is shown as SEQ ID NO:1 under SEQUENCE LISTING. Moreover, the amino acid sequence deduced from the above DNA sequence is shown as SEQ ID NO:2.

Comparison of the DNA sequence thus obtained with that of the decaprenyl diphosphate synthase gene of Saccharomyces cerevisiae as described in Journal of Biological Chemistry, 265, 13157-13164 (1990) revealed about 48% homology on the amino acid level as analyzed using Hitachi Soft Engineering's DNASIS software. Comparison with the decaprenyl diphosphate synthase derived from Schizosaccharomyces pombe as described in Japanese Kokai Publication Hei-9-173076 by means of DNASIS revealed 49% homology on the amino acid level.

(Example 5)

In order to selectively excise the gene region coding for decaprenyl diphosphate synthase from the prepared phage DNA, PCR was carried out using synthetic DNA primers Sa-N1 (which has the sequence of 5'-AACATATGGCCTCACCAGCACTGCGG-3') and Sa-C (which has the sequence of 5'-AAGAATTCCTATCTTGACCTAGTCAACAC-3') in otherwise the same manner as in Example 3. After digestion with the restriction enzymes NdeI and EcoRI, the fragment was inserted into the expression vector pUCNT (disclosed in WO 94/03613) to construct the decaprenyl diphosphate synthase gene expression vector pNTSa1. The restriction map of the expression vector pNTSa1 thus obtained is shown in Fig. 1. It is to be noted that DPS represents the coding region of the decaprenyl diphosphate synthase gene.

(Example 6)

The decaprenyl diphosphate synthase gene expression vector pNTSa1 constructed as above was introduced into Escherichia coli DH5α. The microorganism was shake-cultured in 10 mL of LB broth at 37 ℃ overnight and the cells were harvested by centrifugation (3000 rpm, 20 min.).

The cells were suspended in 1 mL of 3% aqueous solution of sulfuric acid and heat-treated at 120 ℃ for 30 minutes. Then, 2 mL of 14% aqueous solution of sodium hydroxide was added and the mixture was further heat-treated at 120 ℃ for 15 minutes. The lysate obtained was extracted with 3 mL of hexane-isopropyl alcohol (10:2), and after centrifugation, 1.5 mL of the organic layer was separated and evaporated to dryness under reduced pressure. The residue was dissolved in 200 μL of ethanol and a 20 μL portion of the solution was subjected to HPLC analysis (LC-10A, Shimadzu Corporation). Fractionation was carried out using a reversed-phase column (YMC-pack ODS-A, 250 × 4.6 mm, S-5 μm, 120 A) and, as the mobile phase, ethanol-methanol (2:1)

and the coenzyme Q_{10} produced was detected from the absorbance at the wavelength of 275 nm. The result is shown in Fig. 2. As can be seen from Fig. 2, it was found that when the decaprenyl diphosphate synthase gene is introduced into a host and allowed to be expressed, the resulting recombinant Escherichia coli produces coenzyme Q_{10} which E. coli in general inherently does not produce.

The recombinant E. coli DH5α (pNTSa1) obtained as above has been deposited with National Institute of Bioscience and Human-Technology (Higashi 1-1-3, Tsukuba-shi, Ibaraki, Japan) as of Heisei 11, August 17 (accession number FERM BP-6844).

(Example 7)

The octaprenyl diphosphate synthase gene-knockout Escherichia coli KO229 constructed by Kawamukai et al. is known to retain the gene supported on the spectinomycin-resistant plasmid (pKA3) and die on dropout of said plasmid (Journal of Bacteriology, 179, 3058-3060 (1997). The pNTSa1 was introduced into the above knockout strain, culturing the microorganism in 10 mL of ampicillin-containing LB broth by shake culture at 37 ℃ overnight, subculturing 1% of the culture in 10 mL of fresh ampicillin-containing LB broth and culturing the microorganism further by shake-culture at 37 ℃ overnight, and after 9 cycles of the above cultural procedure, selecting the strain growing on ampicillin-containing LB plate medium but not growing on pectinomycin-containing LB plate medium.

(Example 8)

The pNTSa1-transfected strain constructed in Example 7 was shake-cultured in 10 mL of LB broth at 37 ℃ overnight and the cells were harvested by centrifugation (3000 rpm, 20 min.).

The cells were suspended in 1 mL of 3% aqueous solution of sulfuric acid and heat-treated at 120 ℃ for 30 min. Then, 2 mL of 14% aqueous solution of sodium hydroxide was added and the mixture was further heat-treated at 120 ℃ for 15 minutes.

The lysate cells were extracted with 3 mL of hexane-isopropyl alcohol (10:2) and centrifuged to recover 1.5 mL of the organic layer and the solvent was evaporated to dryness under reduced pressure. The residue was dissolved in 200 μL of ethanol and a 20 μL portion of the solution was subjected to HPLC analysis (LC-10A, Shimadzu Corporation). Fractionation was carried out by using a reversed-phase column (YMC-pack ODS-A, 250 × 4.6 mm, S-5 μm, 120 A) and, as the mobile phase, ethanol-methanol (2:1) and the product coenzyme Q_{10} was detected from the absorbance at the wavelength of 275 nm. The result is shown in Fig. 3. It is clear from Fig. 3 that as the decaprenyl diphosphate synthase gene was introduced and allowed to be expressed, the host Escherichia coli was enabled to produce coenzyme Q_{10} which it inherently does not produce and enabled to be transformed so as to yield coenzyme Q_{10} in an increased amount more than that of the coenzyme Q_8 producer E. coli strain.

INDUSTRIAL APPLICABILITY

The gene coding for the key enzyme associated with the biosynthesis of coenzyme Q_{10}, namely decaprenyl diphosphate synthase, was isolated from fungi of the genus Saitoella and its nucleotide sequence was elucidated. Furthermore, the gene was successfully introduced and expressed in Escherichia coli. By utilizing the technology of the invention, coenzyme Q_{10} in use as a pharmaceutical can be produced with improved efficiency.

CLAIMS

1. A DNA of the following (a), (b) or (c):

(a) a DNA having the nucleotide sequence shown under SEQ ID NO:1

(b) a DNA having a nucleotide sequence derived from the nucleotide sequence of SEQ ID NO:1 by the deletion, addition, insertion and/or substitution of one or a plurality of nucleotides

and coding for a protein having decaprenyl diphosphate synthase activity

(c) a DNA which hybridizes with the DNA having the nucleotide sequence of SEQ ID NO:1 under a stringent condition

and codes for a protein having decaprenyl diphosphate synthase activity.

2. A protein of the following (d) or (e):

(d) a protein having the amino acid sequence shown under SEQ ID NO:2

(e) a protein having an amino acid sequence derived from the amino acid sequence of SEQ ID NO:2 by the deletion, addition, insertion and/or substitution of one or a plurality of amino acids and having decaprenyl diphosphate synthase activity.

3. A DNA coding for the protein according to Claim 2.

4. An expression vector constructed by cloning the DNA according to Claim 1 or 3 in an expression vector.

5. The expression vector according to Claim 4 wherein the expression vector is pUCNT.

6. The expression vector according to Claim 5 wherein the expression vector is pNTSa1.

7. A transformant as obtainable by transforming a host microorganism with the DNA according to Claim 1 or 3.

8. A transformant as obtainable by transforming a host microorganism using the expression vector according to Claim 4, 5 or 6.

9. The transformant according to Claim 7 or 8 wherein the host microorganism is Escherichia coli.

10. The transformant according to Claim 9 wherein the Escherichia coli is Escherichia coli DH5 α.

11. The transformant according to Claim 10 which is E. coli DH5α (pNTSa1) (FERM BP-6844).

12. A process for producing a coenzyme Q_{10} which comprises culturing the transformant according to Claim 7, 8, 9, 10 or 11 in a culture broth and harvesting the coenzyme Q_{10} produced and accumulated in the resulting culture.

ABSTRACT

The present invention has for its object to isolate a gene coding for the enzyme synthesizing the coenzyme Q_{10} side chain from a fungal strain of the genus Saitoella and exploit it to advantage for the efficient microbial production of coenzyme Q_{10}.

The present invention provides;

a DNA having the nucleotide sequence shown under SEQ ID NO:1

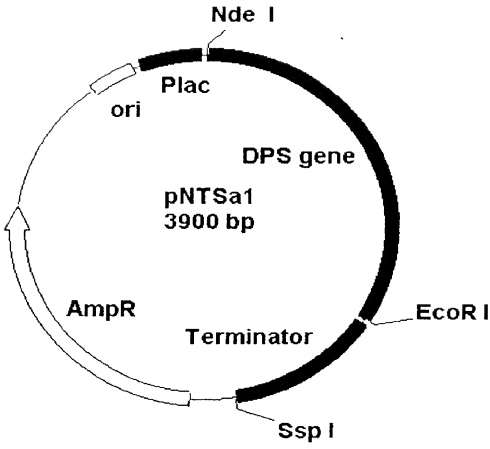
a DNA having a nucleotide sequence derived from the nucleotide sequence of SEQ ID NO:1 by the deletion, addition, insertion and/or substitution of one or a plurality of nucleotides

and coding for a protein having decaprenyl diphosphate synthase activity

a DNA which hybridizes with the DNA having the nucleotide sequence of SEQ ID NO:1 under a stringent condition

and codes for a protein having decaprenyl diphosphate synthase activity.

Fig. 1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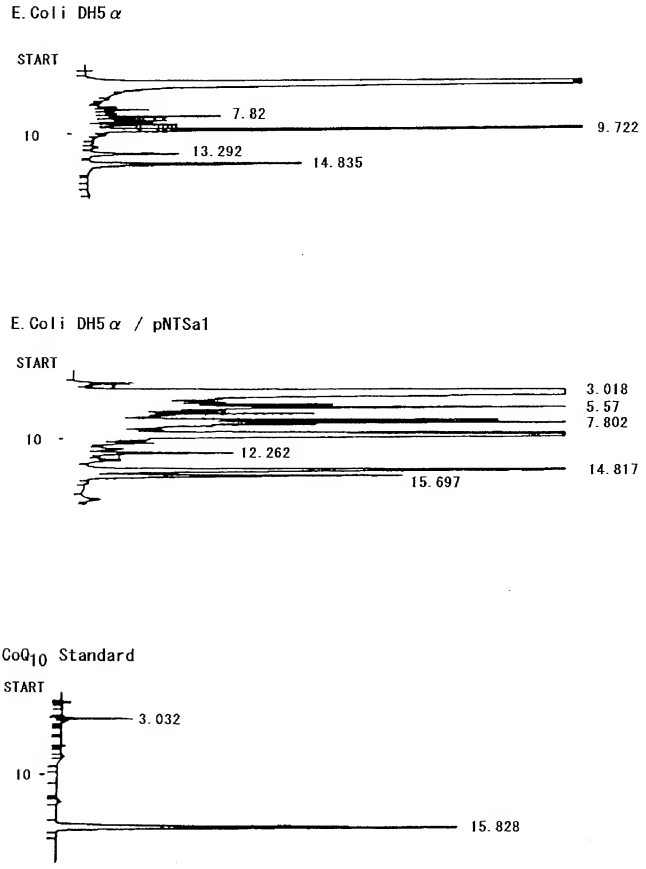
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Fig. 3

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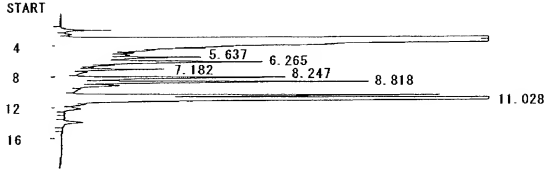

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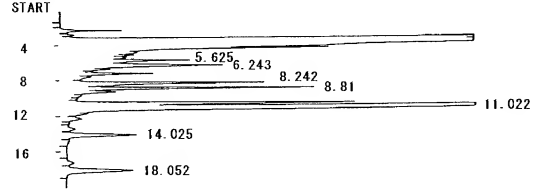

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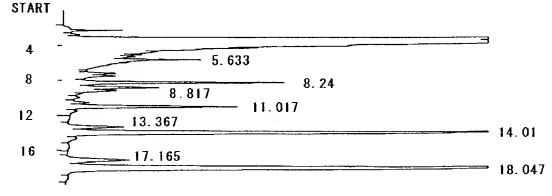
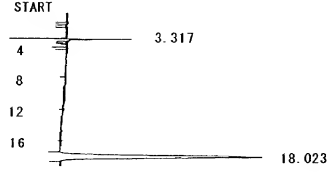


CoQ_{10} Standard



JC03 Rec'd PCT/PTO 25 MAY 2001 ... 339 US
#3

DECLARATION FOR PATENT APPLICATION

1581/00265

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PROCESS FOR PRODUCING COENZYME Q10

the specification of which: (check one)

[ ] is attached hereto. [XX] was filed on August 24, 2000, as United States Patent Application Serial No or PCT International Application Number PCT/JP00/05659, and was amended on __________ 19__ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with 37 CFR § 1.56(a).

Prior Foreign Application(s): I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate listed below, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

			Priority Claimed	
11/237561	Japan	24/August/1999	[XX]	[ ]
(Application No.)	(Country)	(Day/Month/Year Filed)	YES	NO
______	______	______	[ ]	[ ]
(Application No.)	(Country)	(Day/Month/Year Filed)	YES	NO

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date
______	______
______	______

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below or 34 U.S.C. § 365(c) of any PCT International Application designating the United States of America listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application or PCT application in the manner provided by 35 U.S.C. § 112, first paragraph, I acknowledge the duty to disclose material information as defined in 37 CFR § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(U.S. or PCT Application Serial No.)	(U.S. or PCT Filing Date)	(Status - patented, pending, abandoned)
______	______	______

21

I hereby appoint the following registered practitioners: Rudolf E Hutz, Reg. No. 22,397; John D. Fairchild, Reg. No. 19,756; Harold Pezzner, Reg. No. 22,112; Richard M. Beck, Reg. No. 22,580, Paul E. Crawford, Reg. No. 24,397; Burton A. Amernick, Reg. No. 24,852; Morris Liss, Reg. No. 24,510; George R. Pettit, Reg. No. 27,369, Patricia Smink Rogowski, Reg. No. 33,791; Robert G. McMorrow, Jr., Reg. No. 30,962; Ashley I. Pezzner, Reg. No. 35,646; William E. McShane, Reg. No. 32,707; Mary W. Bourke, Reg. No. 30,982; Gerard M. O'Rourke, Reg. No. 39,794; James M. Olsen, Reg. No. 40,408; Francis DiGiovanni, Reg. No. 37,310; Eric J. Evain, Reg. No. 42,517; William E. Curry, Reg. No. 43,572; Daniel C. Mulveny, Reg No. 45,897; John A. Evans, (Agent) 44,100; and Elliot C. Mendelson (Agent), Reg No. 42,878, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Send Correspondence and Direct Telephone Calls to:
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

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DECLARATION FOR PATENT APPLICATION
Page 2

2-00

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6-00

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Citizenship Japanese JPX

Post Office Address Same as above

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